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*Published in:*  
Gynecologic Oncology

*DOI:*  
[10.1016/j.ygyno.2009.09.014](https://doi.org/10.1016/j.ygyno.2009.09.014)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2010

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Duiker, E. W., van der Zee, A. G., de Graeff, P., Boersma-van, E. W., Hollema, H., de Bock, G. H., de Jong, S., & de Vries, E. G. (2010). The extrinsic apoptosis pathway and its prognostic impact in ovarian cancer. *Gynecologic Oncology*, 116(3), 549-555. <https://doi.org/10.1016/j.ygyno.2009.09.014>

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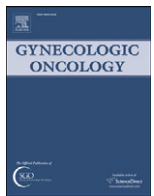
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## The extrinsic apoptosis pathway and its prognostic impact in ovarian cancer

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### ARTICLE INFO

#### Article history:

Received 16 June 2009

Available online 3 December 2009

#### Keywords:

Apoptosis

Ovarian cancer

TRAIL

Prognosis

### ABSTRACT

**Objective.** Death ligand FasL, its agonistic receptor Fas, tumor necrosis factor related apoptosis inducing ligand (TRAIL) and its agonistic death receptors DR4 and DR5 are implied in carcinogenesis, tumor immune surveillance and response to chemotherapy. TRAIL receptor agonists are evaluated as anti-cancer agents. This study aimed to relate expression of death ligands/receptors and downstream initiator caspase 8 and its anti-apoptotic homologue FLICE like inhibitory protein (c-FLIP) in ovarian cancers to chemotherapy response and survival.

**Methods.** Fas, FasL, TRAIL, DR4, DR5, caspase 8 and c-FLIP were determined immunohistochemically on a tissue microarray containing 382 ovarian cancers. Protein expression profiles were correlated with clinicopathologic variables, chemotherapy response and survival.

**Results.** Most tumors expressed DR4, DR5, caspase 8 and c-FLIP. High c-FLIP expression was associated with expression of caspase 8 and both TRAIL receptors. TRAIL and Fas were associated with low tumor grade and better progression-free survival (HR 0.63,  $p = .018$  and HR 0.54,  $p = .012$ ), respectively, and Fas with disease-specific survival (HR 0.49,  $p = 0.009$ ) in univariate analysis.

**Conclusions.** Fas and TRAIL loss is associated with dedifferentiation and worse prognosis. Expression of DR4, DR5, caspase 8 and c-FLIP by most ovarian cancers does not correlate with survival. High c-FLIP expression should be taken into account for death receptor targeted therapies.

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### Introduction

Ovarian cancer is the fifth most common cause of cancer deaths in women [1]. Late stage disease at diagnosis and acquired resistance to chemotherapy are characteristic for the course of most ovarian cancers. These characteristics exemplify the complexity of ovarian carcinogenesis, of which a defined sequence of progression has not yet been established [2]. The resulting heterogeneity among ovarian cancers and consequently in factors underlying clinical response complicates the definition of prognostic and predictive factors for individualized treatment. Distinctive for all cancers is deregulation of the apoptotic machinery [3]. Apoptosis can be induced through two pathways. In the intrinsic pathway, diverse cellular stressors cause sensors within the cell to promote cytochrome c release from the mitochondria, resulting in the formation of the apoptosome and activation of caspase 3, which sets the final execution phase of apoptosis in motion. The extrinsic apoptotic pathway is activated upon binding of death ligands from the tumor necrosis factor (TNF) family to their cognate receptors at the cell surface. The death ligands TNF

related apoptosis inducing ligand (TRAIL) and Fas ligand (FasL/CD95L) are members of the TNF family [4]. TRAIL can bind five receptors of which death receptor 4 (DR4) and death receptor 5 (DR5) transmit an apoptotic signal [5]. FasL binds to one agonistic receptor, Fas (CD95), and one soluble antagonistic receptor, DcR3 [6]. Trimerization of the receptors upon ligand binding causes formation of a death inducing signaling complex (DISC) in which the initiator caspase 8 is activated. Active caspase 8 cleaves various designated cellular proteins including pro-caspase 3, resulting in apoptosis [4]. An important regulator of caspase 8 activation is its anti-apoptotic homologue c-FLIP, which is up-regulated in many tumor types and involved in resistance to chemotherapy and death receptor induced apoptosis [7].

Sensitivity of cancer cells to death ligand induced apoptosis has resulted in development of death receptor targeted drugs as anti-cancer agents. Because systemic administration of Fas targeted agents caused severe hepatotoxicity in mice [8,9], only therapies directed at local administration are now investigated [10]. The recombinant human (rh) form of TRAIL and agonistic antibodies targeting DR4 and DR5 show efficacy in numerous tumor cell lines, including ovarian cancer cell lines and in various xenograft tumor models in mice, without side effects [11]. These results have led to clinical studies which showed that these agents are well tolerated [12–14].

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Considering the development of targeted therapies for TRAIL receptor and Fas activation, assessing expression of key proteins of the extrinsic pathway in ovarian cancer is of interest. Alterations in the expression of FasL, TRAIL, its receptors [15,16], caspase 8 [15,17] and c-FLIP [7] have been implied in carcinogenesis and may hamper future therapies directed at death receptors. Furthermore, because response to chemotherapeutic drugs can be mediated through death ligand dependent and independent activation of caspase 8, these alterations may cause resistance to chemotherapy [18–20]. Robust co-expression data of proteins involved in the extrinsic pathway may define occurrence of these alterations in tumors and their impact on prognosis. Furthermore, they may assist in patient selection for future therapies targeting the extrinsic pathway.

Therefore, the aim of this study was to evaluate protein expression of Fas, FasL, TRAIL, DR4, DR5, caspase 8 and c-FLIP on a tissue microarray (TMA) containing tumor tissue of 382 ovarian cancer patients and to correlate these expression profiles with clinicopathological characteristics and disease outcome.

## Materials and methods

### Patients

From ovarian cancer patients treated since 1985 at the University Medical Center Groningen or affiliated hospitals all clinical, pathological and follow-up data have prospectively been stored in a database. Tumor samples from 382 patients were collected on a TMA. Patients with borderline or non-epithelial tumors were excluded. Primary treatment for all patients consisted of surgery and 90% of the patients eligible for systemic treatment received platin-based regimens as described previously [21]. Primary tumor samples obtained at surgery before any systemic treatment was administered were available for 359 patients. When residual tumor mass was present, response to chemotherapy was determined after three or six cycles based on World Health Organization criteria. Intervention surgery was performed after three chemotherapy cycles and second look surgery after six when indicated. Follow-up lasted up to 10 years. All relevant data were filed in a separate anonymous database in which patients were given unique codes to protect patient identity. Database management was restricted to two people with access to the larger database containing all patients' characteristics. Due to these procedures no additional patient or institutional review board approval was required according to Dutch Law.

### TMA construction

TMAs were constructed as described previously [21]. Representative tumor tissue samples were selected from hematoxylin and eosin stained slides. Four 0.6-mm cores were punched from each donor block and put into 12 recipient paraffin TMA blocks. Each array contained 240 tissue cores, representing 55 tumor samples in quadruplicate and 10 internal controls in duplicate (composed of five tumor, one benign and four non-tumor samples). From each block 4- $\mu$ m sections were cut and mounted on aminopropyltriethoxysilane-treated slides.

### Antibodies

The TMAs were stained with polyclonal goat anti-TRAIL (1:100, clone K-18, Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal goat anti-DR4 (1:100, clone C20, Santa Cruz Biotechnology), polyclonal rabbit anti-DR5 (1:100, clone PC392, Calbiochem, San Diego, CA), monoclonal mouse anti-Fas (1:50, clone CH-11, Upstate Biotechnology, Temecula, CA), polyclonal rabbit anti-FasL (1:100, clone N-20, Santa Cruz Biotechnology), monoclonal mouse anti-caspase 8 (1:100, clone 1C12, Cell Signaling Technology, Danvers,

MA) and monoclonal mouse anti-c-FLIP, detecting both FLIP<sub>L</sub> and FLIP<sub>S</sub> (1:10, clone NF6, Alexis, Lausanne, Switzerland).

### Immunohistochemistry

Staining procedures for all antibodies were performed as described previously [22–25]. After deparaffinization in xylene and re-hydration in ethanol, antigen retrieval was performed by incubation in citrate buffer at 96 °C for DR5 and FasL or high pressure cooking for c-FLIP and caspase 8. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in all slides except those for Fas detection. Avidin/biotin blocking solutions (Vector Laboratories, Burlingame, UK) were applied for DR4, DR5 and TRAIL. Prior to primary antibody incubation, slides were pre-incubated with 1% human AB serum (DR4, DR5 and FasL) or normal rabbit serum (TRAIL). Primary antibody incubation for c-FLIP was overnight; other antibodies were applied for 1 h. c-FLIP staining was detected by incubation with EnVision (DAKO, Glostrup, Denmark), caspase 8 staining with rabbit anti-mouse peroxidase antibody (DAKO), followed by goat anti-rabbit peroxidase antibody (DAKO) and for all other stainings with appropriate biotinylated secondary antibodies and peroxidase-labeled streptavidin (DAKO). Peroxidase activity was visualized with diaminobenzidine. Slides were counterstained with hematoxylin.

Normal tissue (kidney for FasL, liver for Fas) and tumor sections found positive on previous occasions served as positive control for Fas, FasL, DR4, DR5 and TRAIL staining. Negative controls were obtained by omission of the primary antibody, and by incubation with normal isotype controls. For caspase 8 and c-FLIP controls were used as described previously [24].

All sections were simultaneously reviewed by two observers (E.W.D. and W.B.-v.E.), without knowledge of the clinical data. Independent scoring was performed prior to simultaneous evaluation with agreement of >90% for all stainings. Discordant cases and final scoring were reviewed with a gynecological pathologist (H.H.) and assigned on consensus of opinion. Cores containing <10% tumor tissue and all cases with <2 cores were excluded from final analysis. Staining intensity was estimated and scored semi-quantitatively in four classes for DR4, DR5 and caspase 8 as negative (0), moderate (1), positive (2) and strong positive (3). Staining for Fas, FasL, TRAIL and c-FLIP was scored in three classes as negative (0), moderate (1) and positive (2). If heterogeneous staining intensity occurred between four cores of the same tumor, the highest staining intensity was chosen for final scoring if the core with highest staining contained >50% tumor tissue. For statistical analysis all classes were initially studied separately and then dichotomized. For DR4, DR5 and caspase 8, categories 2 and 3 were considered positive and 1 and 0 as negative. For TRAIL, FasL and Fas, 2 was considered positive and 1 and 0 as negative. For c-FLIP staining 2 and 1 were considered positive and 1 as negative [24].

### Statistical analysis

Statistical analysis was performed with SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL). Comparisons between categorical variables were made with  $\chi^2$  tests or Fisher exact tests where appropriate. Comparisons between unpaired tumor samples obtained before and after chemotherapy were made using Mann–Whitney U tests. To exclude the possibility of a type I error in these multiple comparisons, *p* values <0.01 were considered statistically significant. Response to chemotherapy was analyzed using logistic regression analysis for patients with a residual tumor mass  $\geq 2$  cm receiving platinum-based chemotherapy (*n* = 141). Differences in progression-free survival and disease-specific survival were analyzed with two-sided log-rank testing and Cox proportional hazards analysis. *p* values <0.05 were considered significant. Progression-free and disease-specific survival were defined, respectively, as time from primary surgery until date of progression or relapse and as time from primary surgery until death

**Table 1**  
Clinicopathological characteristics of the patients (n = 382).

	n	%
FIGO stage		
Stage I	73	19.1%
Stage II	36	9.4%
Stage III	223	58.4%
Stage IV	47	12.3%
Missing	3	0.8%
Tumor type		
Serous	226	59.2%
Mucinous	46	12.0%
Clear cell	48	12.6%
Endometriod	20	5.2%
Adenocarcinoma NOS	19	5.0%
Other	23	6.0%
Tumor grade		
Grade I	68	17.8%
Grade II	90	23.6%
Grade III	159	41.6%
Undifferentiated	14	3.7%
Missing	51	13.4%
Residual disease		
<2 cm	183	47.9%
≥2 cm	173	45.3%
Missing	26	6.8%
Type of chemotherapy		
No chemotherapy	55	14.4%
Platinum monotherapy	164	42.9%
Platinum/taxane containing	119	31.2%
Other regimen	40	10.5%
Missing	4	1.0%
Age at diagnosis (years)		
Median	58.4	
Range (years)	21.8–89.8	
Follow-up (months)		
Median	29.3	
Range (months)	0–213	

due to ovarian cancer. For multivariate analysis, age at diagnosis (<58 years (median), ≥58 years), FIGO stage (I/II (early), III/IV (late)), tumor type (serous, non-serous), tumor grade (grade I/II, grade III/undifferentiated) and residual tumor size after primary surgery (<2 cm, ≥2 cm) were used as covariates.

**Table 2**  
Staining results and clinicopathological characteristics.

	DR4 (%)	p	DR5 (%)	p	Caspase 8 (%)	p	c-FLIP (%)	p	TRAIL (%)	p	FasL (%)	p	Fas (%)	p
Age														
<58 years	129/169 (76.33)	0.27	159/169 (94.1)	0.62	68/87 (78.2)	0.60	141/166 (84.9)	0.88	33/166 (19.9)	0.25	53/167 (31.7)	0.09	19/166 (11.5)	0.86
>58 years	116/164 (70.7)		161/168 (95.8)		91/112 (81.3)		146/174 (83.9)		26/174 (14.9)		71/174 (40.8)		18/169 (10.7)	
Stage														
Early stage	70/98 (71.4)	0.58	88/93 (94.6)	0.78	73/93 (78.5)	0.70	81/97 (83.5)	0.74	25/97 (25.8)	0.01	40/97 (41.2)	0.26	16/96 (16.7)	0.05
Late stage	174/232 (75)		230/241 (95.4)		198/239 (82.8)		204/240 (85.0)		33/240 (13.8)		82/241 (34.0)		21/236 (8.9)	
Histology														
Serous	148/192 (77.1)	0.10	191/199 (96.0)	0.32	113/144 (78.5)	0.44	168/197 (85.3)	0.65	33/198 (16.7)	0.77	65/197 (33.0)	0.14	20/196 (10.2)	0.60
Non-serous	97/141 (68.8)		129/138 (93.5)		46/55 (83.7)		119/143 (83.2)		26/142 (18.3)		59/144 (41.0)		17/139 (12.2)	
Grade														
Grade I/II	97/137 (70.8)	0.36	131/137 (95.6)	0.61	42/55 (76.4)	0.55	113/139 (81.3)	0.049	34/140 (24.3)	0.006	60/140 (42.9)	0.03	26/139 (18.7)	<0.001
Grade III/ undiff	119/157 (75.8)		150/160 (93.8)		99/122 (81.1)		143/160 (89.4)		19/158 (12.0)		48/149 (30.2)		5/158 (3.2)	
Residual tumor														
<2 cm	113/164 (68.9)	0.10	154/162 (95.1)	1.00	46/58 (79.3)	1.00	135/165 (81.8)	0.38	36/165 (21.8)	0.08	69/165 (41.8)	0.01	22/163 (13.5)	0.02
≥2 cm	114/147 (77.6)		145/153 (94.8)		103/130 (79.2)		131/153 (85.6)		21/153 (13.7)		44/154 (28.6)		8/150 (5.3)	

p values are derived from  $\chi^2$  analysis.

## Results

### Patient characteristics

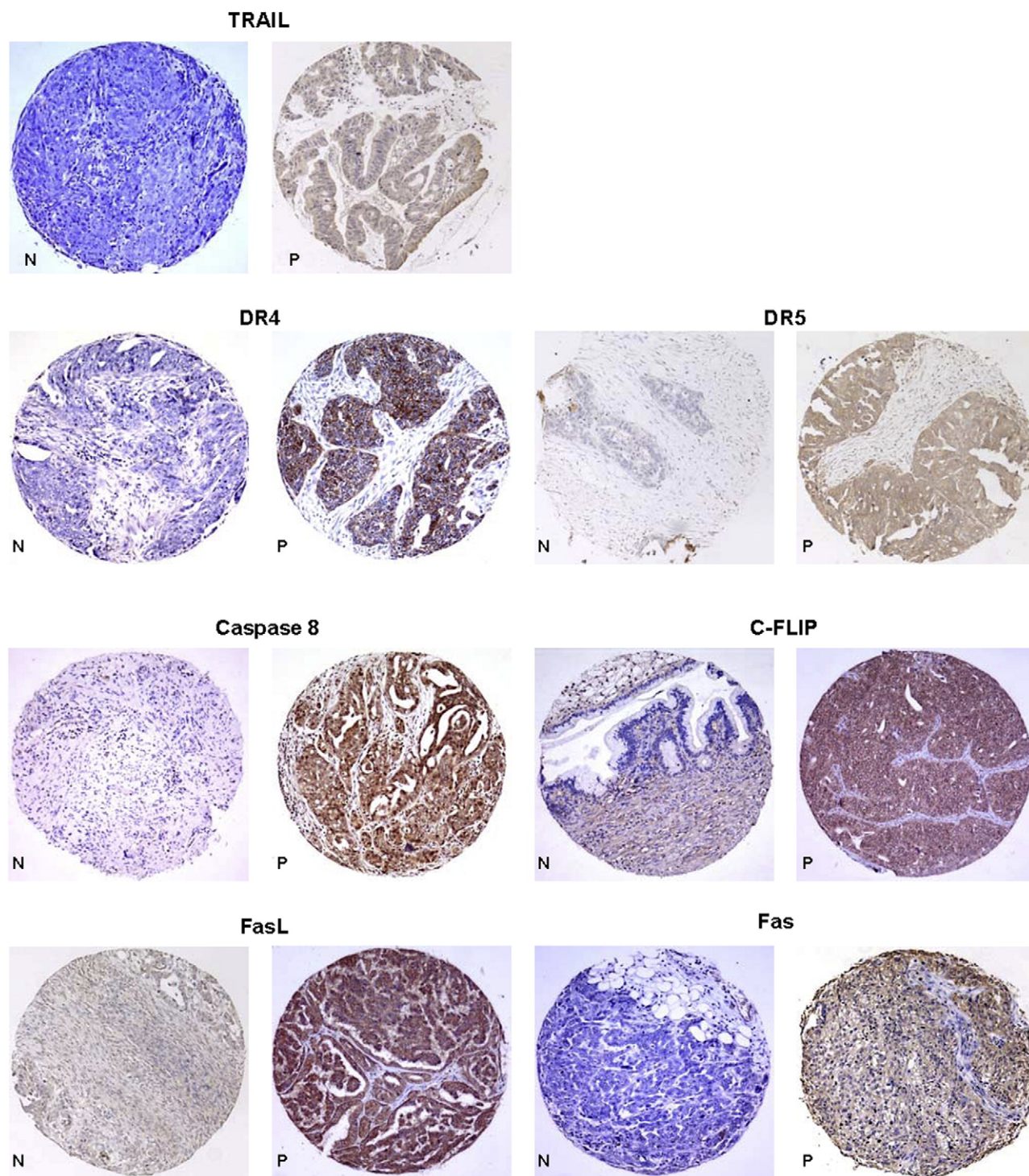
Clinicopathological data are summarized in Table 1. Median follow-up time was 29.3 months (range 0–213); one patient was lost to follow-up. Three hundred seventy-six patients (98.4%) received primary surgery, whereas 6 patients (1.6%) received chemotherapy prior to debulking surgery. Debulking surgery with a residual tumor mass of <2 cm was achieved in 75 (30.5%) late stage patients. Three hundred twenty-three (84.6%) patients received first line chemotherapy, of whom 173 (49.0%) received it as adjuvant therapy with no evidence of residual tumor. In 55 patients no chemotherapy was administered because of stage Ia disease (34 patients, 9%), ineligibility or patient refusal. At the time of data analysis, 22 (20.4%) early and 184 (68.1%) late stage patients had died of ovarian cancer, 3 (2.8%) early and 38 (14.1%) late stage patients were alive with disease and the other patients were alive without evidence of disease. Median progression-free survival was 49.6 (range 0–207) months for early and 11.5 (range 0–149) months for late stage patients. Median disease-specific survival was 57.6 (range 0–207) months and 19.7 (range 0–213) months for early and late stage patients, respectively.

### Associations of proteins with clinicopathological characteristics

Staining results were obtained in 92.8–94.7% of primary tumors available (n = 359) (Table 2). Staining for all proteins was cytoplasmic, with no apparent membranous staining (Fig. 1).

Most tumors expressed DR5, DR4, caspase 8 and c-FLIP (Table 2). Combining data of 322 tumors with expression results on both DRs showed that 70.8% of tumors expressed both death receptors, 26.7% expressed one receptor and only 2.5% expressed neither DR4 nor DR5. Positive c-FLIP expression was associated with higher differentiation grade ( $p = 0.049$ ). TRAIL, Fas and FasL were less frequently expressed. TRAIL expression was more frequent in tumors of low grade ( $p = 0.006$ ) and reduced in late stage tumors ( $p = 0.01$ ). FasL and Fas expression occurred more often in low grade tumors ( $p = 0.03$  and  $p < 0.001$ , respectively) and were associated with a smaller residual tumor mass after primary surgery ( $p = 0.01$  and  $p = 0.02$ ,





**Fig. 1.** Results of immunostaining for TRAIL, DR4, DR5, caspase 8, c-FLIP, FasL and Fas. N stands for negative and P for positive staining (magnification  $\times 10$ ).

respectively). When early and late stage tumors were analyzed separately, TRAIL was not associated with grade, while late stage tumors of low grade expressed FasL and Fas more frequently ( $p=0.02$  and  $p=0.001$ , respectively). After adjustment for multiple testing, only the associations of TRAIL and Fas with low grade tumors sustained.

#### Associations between proteins

Comparison of protein expression profiles of biological relevance revealed several associated profiles ([Supplementary Table 1A and B](#)).

In early stage tumors, DR5 was positively associated with caspase 8 staining ( $p=0.008$ ). In late stage tumors, DR4 staining correlated with positive FLIP staining ( $p=0.001$ ) and negative TRAIL staining ( $p=0.007$ ). Strikingly, 75.7% of the tumors expressing both DRs also expressed c-FLIP, while tumors with reduced expression of at least one DR were more often associated with negative c-FLIP expression (60.5%) ( $p<0.0001$ ) ([Fig. 2](#)). These associations were also observed for early ( $p=0.004$ ) and late stage tumors ( $p=0.001$ ) separately. Caspase 8 staining was positively correlated with c-FLIP ( $p=0.008$ ) in late stage tumors. Finally, in late stage tumors negative Fas expression was associated with negative FasL staining ( $p=0.008$ ). For

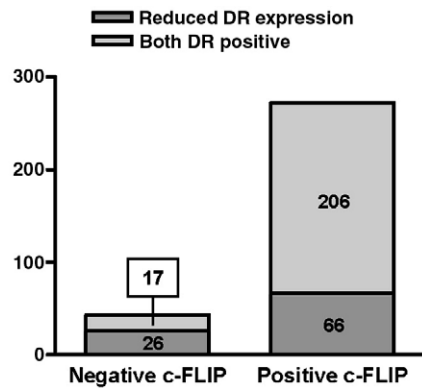


Fig. 2. Death receptor expression in relation to c-FLIP expression in early and late stage ovarian tumors.

188 patients data on p53 staining were available [21]. No relationships were identified between the protein expression of the proteins under study and p53.

#### Protein expression in pre- and post-chemotherapy tumor samples

Comparison of staining patterns in paired tumor samples ( $n = 43$ ) revealed no alterations in protein expression profiles after chemotherapy. When primary tumor samples were compared with all post-chemotherapy samples available, Fas expression was reduced in post-chemotherapy samples ( $p = 0.048$ ).

#### Response to chemotherapy and survival in relation to protein staining

To assess the presence of a correlation between expression of the proteins under study and response to chemotherapy, univariate regression analysis was performed in 141 patients with a residual tumor mass  $\geq 2$  cm after initial surgery, who received platinum-based chemotherapy. Expression profiles were not correlated with response to chemotherapy.

Positive TRAIL expression was associated with a better progression-free survival in log-rank tests and univariate Cox proportional hazard analysis (HR 0.63, 95% CI 0.42–0.92,  $p = 0.018$ ) (Supplementary Table 2). However, this association was not found when the data were analyzed separately in early and late stages (Fig. 3). Positive Fas staining was associated with better progression-free and disease-specific survival ( $p = 0.012$  and  $p = 0.008$ , respectively) (Figs. 4A and B), which was also observed in Cox proportional hazard analyses (HR 0.54, 95% CI 0.33–0.88,  $p = 0.012$  and HR 0.49, 95% CI 0.28–0.84  $p = 0.009$ , respectively). In subgroup analysis Fas was not associated with survival in early and late stage tumors.

In multivariate analysis only advanced stage and a residual tumor  $\geq 2$  cm after primary surgery were independent predictors of poor progression-free survival (HR 3.92, 95% CI 2.17–7.084,  $p < 0.0001$  and HR 1.94, 95% CI 1.33–2.83,  $p = 0.001$ , respectively) and disease-specific survival (HR 3.3, 95% CI 1.73–6.29,  $p < 0.0001$  and HR 2.11, 95% CI 1.41–3.16,  $p < 0.0001$ , respectively).

#### Discussion

In the largest study to date analyzing the protein expression of the death ligands TRAIL, FasL, their cognate agonistic receptors, caspase 8 and c-FLIP in ovarian cancers, we showed that the majority of cancers expressed at least one death receptor, as well as caspase 8 and its anti-apoptotic homologue c-FLIP. Moreover, these data show that derangement of the Fas/FasL system, which is implied in malignant transformation of the ovaries [26] is indeed the case in human ovarian cancer. In addition, ovarian tumors that have retained Fas expression are better differentiated and have a better progression-free and disease-specific survival, which support data showing that loss of Fas expression is implied in dedifferentiation and acquisition of a higher malignant potential in several cancers [27–29]. Previous studies examining protein expression of Fas, FasL or both in ovarian cancers showed substantial variation which can be explained by small sample sizes, inclusion of tumors classified as benign, borderline and malignant and different use of antibodies and scoring systems [23,30–32]. Moreover, in agreement with a previous study [33] we used cut-off values for definition of positive or negative staining based on dichotomization of staining classes according to their association with prognosis.

FasL expression is commonly reported to increase with malignant progression and tumor grade, which was not observed in our study [29,32]. In addition, FasL expression was not associated with a worse prognosis and is therefore not supportive for the tumor counterattack hypothesis [34] in ovarian cancers.

TRAIL expression was associated with lower tumor grade and better progression-free survival when all tumors were analyzed. In previous studies in ovarian cancers TRAIL was also associated with low tumor grade [35] and early stage [22,35], but not with prognosis [22,35,36]. Among colon adenomas and carcinomas loss of TRAIL expression occurred in a subset of colon carcinomas [37] and in samples spanning oral cancer progression it was an early event in carcinogenesis [38]. These results suggest that loss of TRAIL expression represents a survival advantage for tumor cells, possibly because they evade apoptosis induction by para- or autocrine released TRAIL. This is supported by a study which showed that in response to interferon-gamma Ewing tumor cells produce and secrete functional TRAIL that induces apoptosis in unstimulated Ewing tumor cells [39].

The majority of tumors in our study expressed DR4, DR5, caspase 8 and c-FLIP. A striking finding was the association of c-FLIP

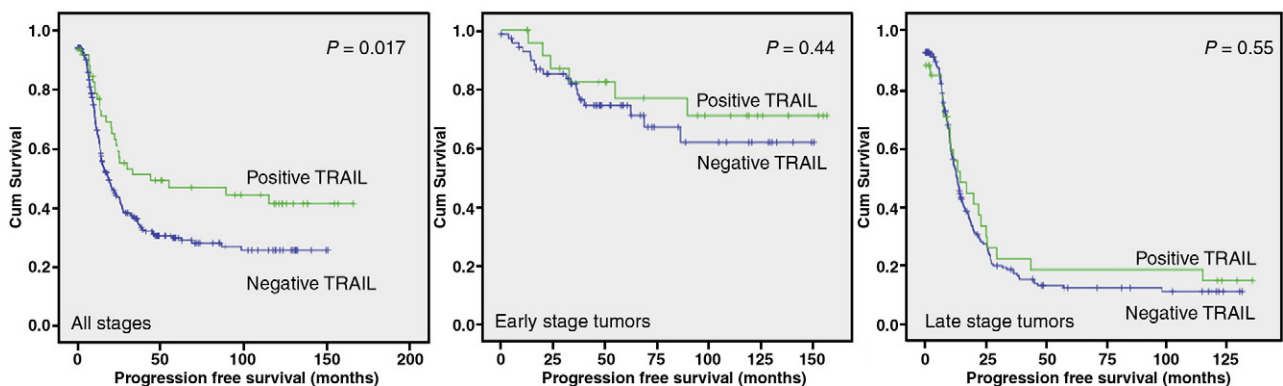
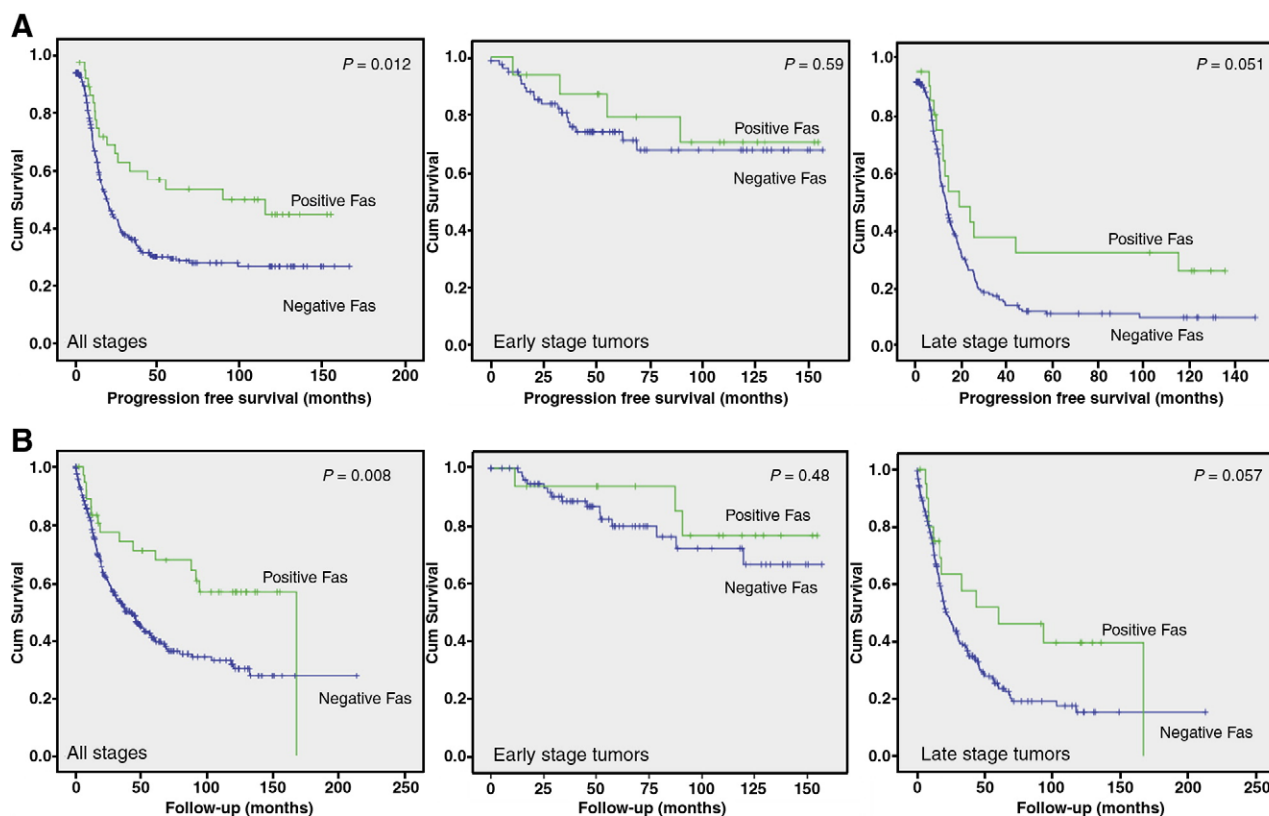


Fig. 3. Progression-free survival according to TRAIL expression in all patients and early and late stage tumors separately.





**Fig. 4.** (A) Progression-free survival according to Fas expression in all patients and early and late stage tumors separately. (B) Disease-specific survival according to Fas expression in all patients and early and late stage tumors separately.

expression with expression of both death receptors and with caspase 8, which suggests that apoptotic death receptor signaling is counteracted in ovarian cancers. These associations were, however, not correlated with prognosis, nor were the individual proteins. Up-regulation of the anti-apoptotic caspase 8 homologue c-FLIP was reported in several tumor types [7] and was associated with a poor clinical outcome in Burkitt lymphomas [40] and bladder urothelial carcinomas [41]. In vitro, c-FLIP induces resistance to Fas and TRAIL receptor targeted drugs in vitro [42–45] and is therefore a target for modulating death receptor induced apoptosis. In colon cancer patients, high DR4 expression was an independent prognostic factor for worse disease-free and overall survival [46]. High DR5 expression was associated with decreased survival in univariate analysis in ovarian cancers [35] and was independently associated with decreased survival in breast and small lung cancers [47,48]. These different results underline the complexity of death receptor signaling, which is not only dependent on expression of its constituents, but also on external factors and the intracellular apoptotic machinery and might therefore be tissue specific. Moreover, it becomes increasingly evident that single prognostic factors, e.g., HER2 and hormone receptors in breast and c-kit in GIST tumors, are rather an exception than the rule. Considering the redundancy of signaling pathways, it is not surprising that in most tumors numerous factors are likely to influence prognosis [49]. Furthermore, although alterations in the death receptor pathway are involved in chemoresistance [19,50,51], the main tumoricidal mechanism of most conventional drugs is not likely to act through the extrinsic pathway. Therefore, our results show that in ovarian cancers loss of TRAIL and Fas expression represents an important aspect in dedifferentiation and escape from tumor immune surveillance. In addition, deregulation of the extrinsic pathway by c-FLIP expression occurs, but these changes are not of critical significance for disease outcome. They may, however, be of significance for future therapies targeting the extrinsic pathway. Clinical studies with rhTRAIL,

agonistic antibodies directed at DR4 or DR5 and Fas are ongoing. Membranous DR expression on tumors is a pre-requisite for these drugs to be effective as anti-cancer agents, but functionality of the downstream signaling pathway is of equal importance. Therefore, it needs to be established whether these protein expression profiles correlate with functionality of the death receptor pathway in ovarian cancers, which can be achieved by relating clinical responses to TRAIL receptor agonists with tumor characteristics.

Resistance to death receptor targeted agents and to conventional chemotherapies can be overcome by combining these drugs. Many different mechanisms were described to be involved in this synergy, including down-regulation of c-FLIP [52]. Consequently, combinations of conventional chemotherapeutics and death receptor drugs warrant further development as novel strategies for cancer treatment.

In conclusion, loss of Fas and TRAIL is associated with dedifferentiation and a worse prognosis in ovarian cancers. Expression of anti-apoptotic c-FLIP is associated with caspase 8 and death receptor expression, which should be considered for future death receptor targeted therapies in ovarian cancer.

#### Conflict of interest statement

The authors declare that there are no conflicts of interest.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ygyno.2009.09.014](https://doi.org/10.1016/j.ygyno.2009.09.014).

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